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## **No benefit of therapeutic vaccination in clinically healthy cats persistently infected with feline leukemia virus**

Helfer-Hungerbuehler, A Katrin ; Spiri, Andrea Monika ; Riond, Barbara ; Grest, Paula ; Boretti, Felicitas S ; Hofmann-Lehmann, Regina

**Abstract:** Therapeutic vaccinations have a potential application in infections where no curative treatment is available. In contrast to HIV, efficacious vaccines for a cat retrovirus, feline leukemia virus (FeLV), are commercially available. However, the infection is still prevalent, and no effective treatment of the infection is known. By vaccinating persistently FeLV-infected cats and presenting FeLV antigens to the immune system of the host, e.g., in the form of recombinant and/or adjuvanted antigens, we intended to shift the balance toward an advantage of the host so that persistent infection could be overcome by the infected cat. Two commercially available FeLV vaccines efficacious in protecting naïve cats from FeLV infection were tested in six experimentally and persistently FeLV-infected cats: first, a canarypox-vectored vaccine, and second, an adjuvanted, recombinant envelope vaccine was repeatedly administered with the aim to stimulate the immune system. No beneficial effects on p27 antigen and plasma viral RNA loads, anti-FeLV antibodies, or life expectancy of the cats were detected. The cats were unable to overcome or decrease viremia. Some cats developed antibodies to FeLV antigens although not protective. Thus, we cannot recommend vaccinating persistently FeLV-infected cats as a means of improving their FeLV status, quality of life or life expectancy. We suggest testing of all cats for FeLV infection prior to FeLV vaccination.

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1 **No benefit of therapeutic vaccination in clinically**  
2 **healthy cats persistently infected with feline leukemia**  
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4

5 **A. Katrin Helfer-Hungerbuehler<sup>1§</sup>, Andrea M. Spiri<sup>1</sup>, Barbara Riond<sup>1</sup>, Paula**  
6 **Grest<sup>2</sup>, Felicitas S. Boretti<sup>3</sup> and Regina Hofmann-Lehmann<sup>1</sup>**  
7

8 <sup>1</sup>Clinical Laboratory and Center for Clinical Studies, Vetsuisse Faculty, University of  
9 Zurich, Zurich, Switzerland

10 <sup>2</sup> Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich,  
11 Switzerland

12 <sup>3</sup> Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich,  
13 Zurich, Switzerland  
14

15 <sup>§</sup>Corresponding author  
16

17 Mailing address: Clinical Laboratory, Vetsuisse Faculty, University of Zurich,  
18 Winterthurerstr. 260, 8057 Zurich, Switzerland.

19 Phone: +41 44 635 82 86. Fax: +41 44 635 89 23.

20 E-mail: [khungerbuehler@vetclinics.uzh.ch](mailto:khungerbuehler@vetclinics.uzh.ch)  
21

22 E-mail addresses:

23 AKH: [khungerbuehler@vetclinics.uzh.ch](mailto:khungerbuehler@vetclinics.uzh.ch)

24 AS: [aspiri@vetclinics.uzh.ch](mailto:aspiri@vetclinics.uzh.ch)

25 BR: [briond@vetclinics.uzh.ch](mailto:briond@vetclinics.uzh.ch)

26 PG: [grest@vetpath.uzh.ch](mailto:grest@vetpath.uzh.ch)

- 27 FSB: [fboretti@vetclinics.uzh.ch](mailto:fboretti@vetclinics.uzh.ch)
- 28 RHL: [rhofmann@vetclinics.uzh.ch](mailto:rhofmann@vetclinics.uzh.ch)
- 29

## **Abstract**

Therapeutic vaccinations have a potential application in infections where no curative treatment is available. In contrast to HIV, efficacious vaccines for a cat retrovirus, feline leukemia virus (FeLV), are commercially available. However, the infection is still prevalent, and no effective treatment of the infection is known. By vaccinating persistently FeLV-infected cats and presenting FeLV antigens to the immune system of the host, e.g., in the form of recombinant and/or adjuvanted antigens, we intended to shift the balance toward an advantage of the host so that persistent infection could be overcome by the infected cat. Two commercially available FeLV vaccines efficacious in protecting naïve cats from FeLV infection were tested in six experimentally and persistently FeLV-infected cats: first, a canarypox-vectored vaccine, and second, an adjuvanted, recombinant envelope vaccine was repeatedly administered with the aim to stimulate the immune system. No beneficial effects on p27 antigen and plasma viral RNA loads, anti-FeLV antibodies or life expectancy of the cats were detected. The cats were unable to overcome or decrease viremia. Some cats developed antibodies to FeLV antigens although not protective. Thus, we cannot recommend vaccinating persistently FeLV-infected cats as a means of improving their FeLV status, quality of life or life expectancy. We suggest testing of all cats for FeLV infection prior to FeLV vaccination.

## **Keywords**

FeLV; retrovirus; therapeutic vaccination; cat.

51

52 **Abbreviations**

53 EDTA, Ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent  
54 assay; FeLV, Feline Leukemia Virus; HBV, hepatitis B virus; HCV, hepatitis C  
55 virus; TNA, total nucleic acid;

56

## 1. Introduction

Treatment of many chronic infectious diseases remains a challenge. Present curative treatments are often either nonexistent (e.g., HIV/AIDS), inefficient (e.g., hepatitis B virus [HBV] infection) or remain toxic (e.g., IFN- $\alpha$  tolerance in hepatitis C virus [HCV] treatment)[1]. Thus, new classes of therapeutics are being developed, including therapeutic vaccines. Therapeutic vaccines have the potential to act in areas where no optimal treatment has been identified. Their underlying technology can be peptide or protein-based, vector-based (DNA, virus or bacteria-derived vectors), whole cell-based, or antigen-antibody-based. Their range of potential applications is enormous and includes areas such as oncology and infectious diseases, such as HBV and HCV, papillomaviruses or tuberculosis. Most of these therapeutic vaccines are currently in clinical trials (phase 1 and 2), but some have already reached the market. Naïve cats are able to mount a successful immune response after vaccination against FeLV and, in the majority of cats, also after natural infection. This outcome has been described as abortive or regressive infection; the cats show no viremia (abortive) or only transient viremia and low proviral and viral RNA loads (regressive). However, in about a third of the unvaccinated cats exposed to FeLV the immune system fails to develop a successful FeLV-specific response, and the cat develops a persistent infection with fatal outcome due to subsequent FeLV-associated diseases [2-4]. Persistent infection is characterized by persistence of antigenemia and high provirus and plasma viral RNA loads. By vaccinating clinically healthy, persistently infected cats and presenting the FeLV antigens to the immune system (e.g., in the form of recombinant and/or adjuvanted antigens), we intended to shift the balance, at least temporarily, toward an advantage of the host, so that persistent infection can be overcome by the infected cat.

To stimulate the immune system, two commercially available vaccines were chosen which were both shown to be highly efficacious in preventing FeLV infections [5-8]. They differ in their composition and mode of action: the first, Purevax® FeLV, is a recombinant, canarypox-vectored live vaccine and is assumed to stimulate the cellular immune system through activation of phagocytes, antigen-specific cytotoxic T-lymphocytes and the release of cytokines in response to an antigen; the second, Leucogen®, an adjuvanted recombinant envelope protein vaccine, was associated with the induction of a strong humoral immune response. We hypothesize that through stimulation of the immune system by therapeutic vaccination, the boost of the immune system may lead to a reduction of the virus load either through antibody-mediated action or through the cellular immune response. Thus, it was the aim of this study to repeatedly vaccinate persistently FeLV-infected cats and follow the presence and quantity of plasma viral RNA, p27 antigen and anti-FeLV antibody levels.

## **2. Materials and methods**

### **2.1. Sample collection from experimentally infected domestic cats**

All six specified pathogen-free (SPF) cats included in this study were in experimental studies officially approved by the veterinary office of the Swiss Canton of Zurich (11/2011, 160/2010 and 251/2013). The cats were kept in groups under optimal ethological conditions in a barrier facility, as previously described [9]. They had been experimentally infected with FeLV-A/Glasgow-1 within the above mentioned studies [10, 11] between the ages of 10 to 21 weeks (median age = 18 weeks); 0.7 to 1.5 years after infection therapeutic vaccination was started (median duration of infection = 1.5 years). Because there were six cats available with persistent infection at the time, all cats were assigned to the treatment group, while a historical control group was used for comparison of the p27 antigen levels as it had been reported in a previous study

[11]. The five cats in the control group were matched with the cats in the treatment group for duration of FeLV infection (median infection duration at the time point of the start of this study = 1.6 years); moreover, the age at the time point of experimental infection in the cats of the control group was matched to that of the treatment group (median age = 18 weeks)[12]. Identical methods were used for the analysis of samples from cats in the treatment and the control group. EDTA-anticoagulated whole blood samples were collected at three to four week intervals from all cats in the treatment group and at seven to nine week intervals in the historical control group. The general condition of the cats was monitored daily by the animal care takers and thorough clinical examinations were performed weekly by veterinarians. Complete hemograms were performed using a Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan) [13]. For the white blood cell differential, microscopic blood smear evaluation was performed, as described [14].

Three cats (CK1, CK3 and BZ3) had to be euthanized for humane reasons during the vaccination and follow-up period and underwent necropsy and histopathological examination.

## **2.2. FeLV vaccination**

Two types of FeLV vaccines were tested in the current study. First, the cats in the treatment group were vaccinated subcutaneously five times with a non-adjuvanted canarypox-vectored live vaccine (ALVAC) containing FeLV-A *env*, *gag* and part of *pol* (Purevax® FeLV, Biokema S.A., Crissier, Switzerland) at three week intervals. The cats were then followed for another 13 weeks. Then, the cats were vaccinated with a second vaccine, an adjuvanted recombinant protein vaccine containing the envelope glycoprotein of FeLV-A expressed in *Escherichia coli* (Leucogen®, Virbac, Glattbrugg, Switzerland). The non-glycosylated recombinant protein includes the



entire exterior envelope protein (p45) plus the first 34 amino acids of the transmembrane protein p15E [6]. Again, all cats were vaccinated five times at three to four week intervals and subsequently followed for another 13 weeks. The vaccination intervals were chosen according to the manufacturers recommendations ([www.emea.europa.eu](http://www.emea.europa.eu)).

### 2.3. Serological assays

The level of FeLV antigenemia was determined by p27 antigen sandwich ELISA as described [15]. Values above 4% of the positive control run with each assay were considered positive [7]. Anti-FeLV whole virus antibodies were measured by ELISA using 500 ng of gradient purified antigen per well, as described previously [16, 17]. Antibodies to recombinant p15E and the non-glycosylated p45 *env* gene product were measured by ELISA as described [5, 18]. The antibody levels were expressed in relation to a positive and negative control that was run on each plate; for calculation of standardized optical density values see [18].

### 2.4. Nucleic acid extraction

Total nucleic acid (TNA) was extracted from 200 µl EDTA plasma using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Rotkreuz, Switzerland), and the TNA was analyzed for the presence of viral RNA by real-time TaqMan RT-PCR, as described previously [19]. Negative controls consisting of 100 µl of phosphate buffered saline (PBS) were prepared with each batch to monitor cross-contamination.

### 2.5. Statistics

Statistical analyses were performed using GraphPad Prism for Windows, Version 4.03 (GraphPad software, San Diego, CA). The time course of p27 levels, plasma viral

RNA loads, FeLV specific antibodies and hematological parameters within a group were analyzed using the non-parametric Friedman test for paired samples ( $p_F$ ) followed by the Dunn's Multiple Comparison test ( $p_D$ ). The frequency of cats that had to be euthanized during the study in the different groups was compared using the Fisher's exact test ( $p_{\text{Fisher}}$ ). A p-value less than 0.05 was considered significant.

### 3. Results and discussion

The first round of therapeutic vaccination was initiated by repeatedly vaccinating the six persistently FeLV-infected cats with a recombinant, canarypox-vectored vaccine. Virus blood loads were followed using p27 antigen ELISA and real-time TaqMan RT-PCR for plasma viral RNA. During the first vaccination and follow-up period, p27 antigen levels did not show a decrease (Figure 1A). In contrast, 18 and 23 weeks after the first vaccination, FeLV antigen levels had significantly increased compared to week 0 (Figure 1A). No significant changes in antigen levels were detected in FeLV-infected historical controls matched with the treated group for weeks after experimental infection (Figure 1B). Plasma viral RNA loads in the treated group remained stable during the entire observation period (Figure 1C). Prior to the experiment, all cats had undetectable levels of antibodies to FeLV whole virus and the recombinant p45 *env* gene product (Figure 2A and B). Vaccination did not lead to an increase of these FeLV specific antibodies; they remained at background levels ( $\leq 1.5\%$ ) at all times in all cats (Figure 2A and B). This is in accordance with previous observations in naïve cats where vaccination with the canarypox-vectored vaccine did not lead to production of anti-FeLV specific antibodies; although the cats were subsequently efficaciously protected from FeLV challenge infection [7, 8]. Alternatively, if antibodies were produced in the persistently infected cats, they might have been bound *in vivo* by the circulating virus. Antibodies to p15E were low in all

182 cats (< 27%); lowest levels were found in cats CK1 and CK3 (Figure 2C). A slight  
183 overall decrease of antibodies to p15E was found during vaccination with the  
184 canarypox vectored vaccine ( $p_F = 0.0379$ ; Figure 2C). Hematological parameters,  
185 such as hematocrit, leucocyte count, and neutrophil and eosinophil granulocyte counts  
186 did not show any significant changes over time, although some cats had developed  
187 anemia (hematocrit <33%), leukopenia and/or neutropenia (Supplementary Figure 1).  
188 Moreover a significant decrease ( $p_F = 0.0029$ ) was found in lymphocyte counts in  
189 treated cats between week 3 and 25 after the first vaccination ( $p_D < 0.05$ ;  
190 Supplementary Figure 1B). Hematological results of the historical control cats were  
191 not available. One cat (CK1) displayed severe lymphopenia (340 cells/ $\mu$ l) and had to  
192 be euthanized for humane reasons during the first part of the experiment in week 10.  
193 The cat had shown increasing p27 levels (185%, Figure 1A) and no anti-FeLV  
194 specific antibodies prior to clinical deterioration (Figure 2). The pathological  
195 examination revealed that this cat had developed a T-cell lymphoma in the  
196 mediastinum. Lymphoma is a fatal disease frequently associated with FeLV infection  
197 [20, 21]. Thirteen weeks after the last vaccination with the canarypox-vectored  
198 vaccine, a second cat (CK3) had to be euthanized for humane reasons prior to the start  
199 of the second experiment. Again this cat had shown increasing p27 levels (121% at  
200 the time of euthanasia in week 25, data not shown) and undetectable anti-FeLV  
201 antibodies (Figure 2). The cat had developed chronic kidney disease leading to  
202 azotemia and anemia (3619 reticulocytes/ $\mu$ l; Supplementary Figure 1); upon necropsy  
203 severe bilateral membranous glomerulonephritis most likely associated with FeLV  
204 infection was diagnosed. Overall, the first vaccination series with the canarypox-  
205 vectored vaccine did not show any beneficial effects on the presence of virus, anti-  
206 FeLV antibodies, clinical parameters or health status of the cats.

207 The second round of repeated vaccinations using an adjuvanted recombinant protein  
208 vaccine was started with the remaining four cats. No significant changes in FeLV  
209 loads (p27 antigen and plasma viral RNA) were found during the second part of the  
210 study in the treated cats (Figure 3). Prior to the vaccinations, all cats had undetectable  
211 levels of antibodies to FeLV whole virus and the recombinant p45 *env* gene product  
212 (Figure 4A and B). During vaccinations with the recombinant envelope protein  
213 vaccine, a significant increase of antibodies to p45 was detected (week 0 to 28:  $p_F =$   
214 0.0137; week 0 to 19:  $p_F = 0.0069$ ; Figure 4B). To the best of our knowledge this is  
215 the first demonstration of significant antibody production to an adjuvanted FeLV  
216 envelope protein vaccine in long-term persistently FeLV-infected cats. One of the cats  
217 (BP2) developed levels of antibody approaching those observed in naïve cats (Figure  
218 4B) vaccinated with the recombinant *env* vaccine [5], the remaining cats developed  
219 lower antibody levels. This is similar to what had been found in an earlier study,  
220 where persistently FeLV-infected cats were vaccinated against rabies: those cats  
221 developed neutralizing antibodies to the rabies vaccines although to a lower level than  
222 FeLV uninfected cats [22]. Moreover, cats persistently infected with FeLV developed  
223 antibodies to synthetic peptides (TGAL, tetanus toxoid) but delayed and to much  
224 lower levels compared with FeLV uninfected cats [23, 24]. Low antibody levels  
225 against p15E were found (< 20% of the positive control) and no significant change  
226 could be observed during the study period in antibodies to p15E. In addition, the  
227 hematological parameters did not show any significant changes in the vaccinated cats  
228 during the second set of vaccinations and in the 13 weeks thereafter; some cats were  
229 anemic, leukopenic, neutropenic and/or lymphopenic (Supplementary Figure 2 and  
230 data not shown). The differences between the developments of lymphocyte counts  
231 during the two vaccination studies might be the result of the different modes of

actions of the two vaccines (primarily cellular versus humoral immune response). Of note, the statistical power in the second set of experiments was smaller than in the first one due to a smaller number of cats in the treated group. Historical control samples matched for weeks after experimental FeLV infection did not show any significant changes in p27 levels during the time frame observed (Figure 3B). One cat (BZ3) had to be euthanized six weeks after the last vaccination with the recombinant protein vaccine, and necropsy revealed T-cell lymphoma in the mediastinum, pericardium, tonsils and prescapular lymph nodes with secondary leukemia. While antibody levels against p15E were undetectable in the two cats that had to be euthanized earlier (CK1 and CK3), only very low levels were found in the third cat, BZ3, which had to be euthanized due to FeLV-associated disease ( $\leq 3.7\%$  during the second round of repeated vaccinations; Figure 4C). This may indicate that low antibody levels against FeLV p15E might be an indicator of declining health in persistently FeLV-infected cats. In parallel, p27 levels showed a marked increase in the two previously and this third euthanized cat. In agreement with this in a recent study it was found that persistently FeLV-infected cats with fatal FeLV-associated disease had significantly higher blood p27 levels compared to healthy persistently FeLV-infected cats (unpublished observation). Thus, monitoring of these two parameters - blood p27 levels and anti-FeLV p15E antibodies - may be helpful prognostic parameters in persistently FeLV-infected cats.

At the end of the experiment, 2.6 years after FeLV exposure, three of the initial six persistently FeLV-infected vaccinated cats had survived, while in the historical control group five out of five cats had survived during the same timeframe after infection ( $p_{\text{Fisher}} = 0.2$ ). Although there is no significant difference between the two groups in survival of the cats, as a precaution, we recommend not vaccinating

persistently infected cats, and in accordance with the manufacturers, we suggest testing of all cats for FeLV infection prior to FeLV vaccination. Of note, at the time point of submission of this article, the three remaining persistently infected cats were still alive (3.1 years after FeLV infection).

Our results indicate that vaccination of cats persistently infected with FeLV – at least at the time point chosen, 1.5 years after FeLV infection - does not improve the health status of the cats or decrease the virus load. No improvement in the vital parameters was observed in any of the cats. Thus, the authors refrained from initiating larger studies with more cats or analyzing additional parameters investigating e.g., the cellular immune response in these cats.

In contrast to our data, several therapeutic vaccinations have been applied successfully in human medicine, e.g., against cancer: Provenge® vaccine, which aims at treating prostate cancer was the first FDA approved therapeutic cancer vaccine [25]. Furthermore, therapeutic vaccination also against a virus, the human papilloma virus, demonstrated significant overall response rates and DNA-based viral clearance in phase II trials [1]. Similar to our study, and despite over 15 years of intensive research, no effective therapeutic vaccine has been developed against the human retrovirus HIV and AIDS. Although numerous phase I and II trials are ongoing or have already been completed, overall these vaccines have not yet yielded convincing data [1]. However, it needs to be noted that also no efficacious prophylactic vaccines are available against HIV, while very successful vaccines have been commercially available against FeLV for several decades [5, 7, 26].

## 4. Conclusion

Repeated vaccination with a canarypox-vectored vaccine or an adjuvanted recombinant protein vaccine did not shown any beneficial effect on p27 levels, viral RNA loads, anti-FeLV antibodies or survival of the cats. Therefore, we cannot recommend vaccinating persistently FeLV-infected cats as a means of improving their FeLV status, quality of life or life expectancy. Moreover, it seems advisable to test cats for the presence of viremia/antigenemia prior to FeLV vaccination.

## Conflict of interest

The authors declare that they have no conflicts of interest.

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389

## 390 **Figures**

391 **Figure 1 - p27 antigen and plasma viral RNA loads of persistently FeLV**  
392 **infected cats after repeated FeLV vaccination with a recombinant, canarypox-**  
393 **vectored vaccine.** Persistently FeLV infected cats were vaccinated five times (grey  
394 box; arrows). A) FeLV p27 antigen levels in the peripheral blood of vaccinated cats,  
395 B) FeLV p27 antigen levels in the peripheral blood of historical controls matched for  
396 weeks after experimental infection, C) plasma viral RNA loads of vaccinated cats.  
397 FeLV p27 antigen levels and viral RNA loads were tested for significant differences  
398 by the non-parametric Friedmann test (pF) including only paired samples and the  
399 Dunn's Multiple Comparison test: \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

400 **Figure 2 - Antibody levels of persistently FeLV infected cats after repeated**  
401 **FeLV vaccination with a recombinant, canarypox-vectored vaccine.** A)  
402 Antibodies to FeLV whole virus, B) to p45 and C) to p15E as determined by ELISA.  
403 Time points of vaccination are indicated (grey box; arrows). Significant differences  
404 were determined with the non-parametric Friedmann test (pF), including only paired  
405 samples and the Dunn's Multiple Comparison test: no significant differences could be  
406 detected. The cats CK1 and CK3 had to be euthanized due to humane reasons  
407 during and after the first part of the experiment in week 10; and week 25,  
408 respectively.

409

410 **Figure 3 - p27 antigen and plasma viral RNA loads of persistently FeLV**  
411 **infected cats after repeated FeLV vaccination with a recombinant protein**  
412 **vaccine.** Persistently FeLV infected cats were vaccinated five times (grey box;

arrows). A) FeLV p27 levels in the peripheral blood of vaccinated cats, B) FeLV p27 antigen levels in the peripheral blood of historical controls matched for weeks after experimental infection, C) plasma viral RNA loads in the vaccinated cats. FeLV p27 antigen levels and viral RNA loads were tested for significant differences by the non-parametric Friedmann test (pF) including only paired samples and the Dunn's Multiple Comparison test: no significant differences could be detected.

**Figure 4 - Antibody levels of persistently FeLV infected cats after repeated FeLV vaccination with a recombinant protein vaccine.** A) Antibodies to FeLV whole virus, B) to p45 and C) to p15E as determined by ELISA. Time points of vaccination are indicated (grey box; arrows). Significant differences were determined with the non-parametric Friedmann test (pF), including only paired samples and the Dunn's Multiple Comparison test: \* =  $p < 0.05$ . The cat BZ3 had to be euthanized due to humane reasons in week 21.

## Supplementary data

**Supplementary Figure 1 – Time course of hematological parameters of persistently FeLV infected cats after repeated FeLV vaccination with a canarypox-vectored vaccine.**

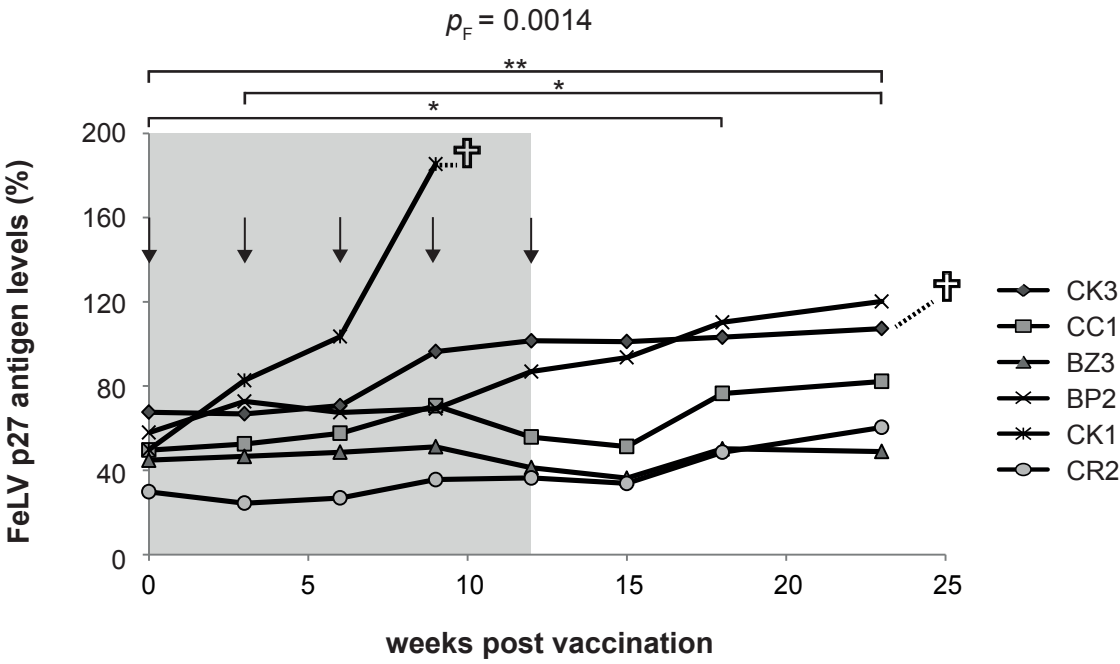
A) Hematocrit. B) Lymphocyte counts. Time points of vaccination are indicated by arrows, the reference ranges (5<sup>th</sup> to 95<sup>th</sup> percentiles) by shaded areas.

**Supplementary Figure 2 – Time course of hematological parameters of persistently FeLV infected cats after repeated FeLV vaccination with a recombinant protein vaccine.**

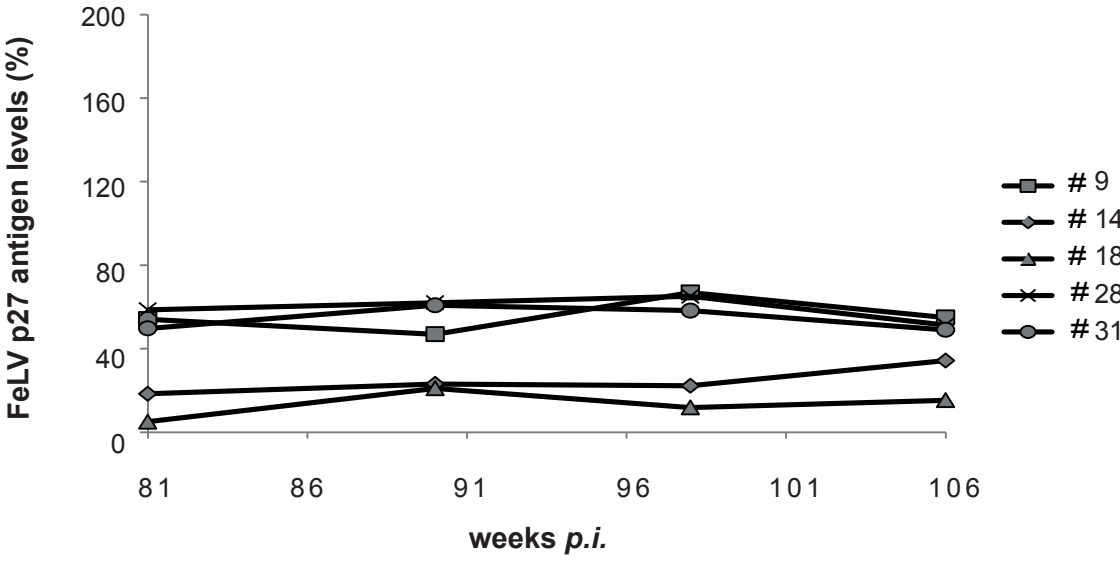
A) Hematocrit. B) Lymphocyte counts. Time points of vaccination are indicated by arrows, the reference ranges (5<sup>th</sup> to 95<sup>th</sup> percentiles) by shaded areas.



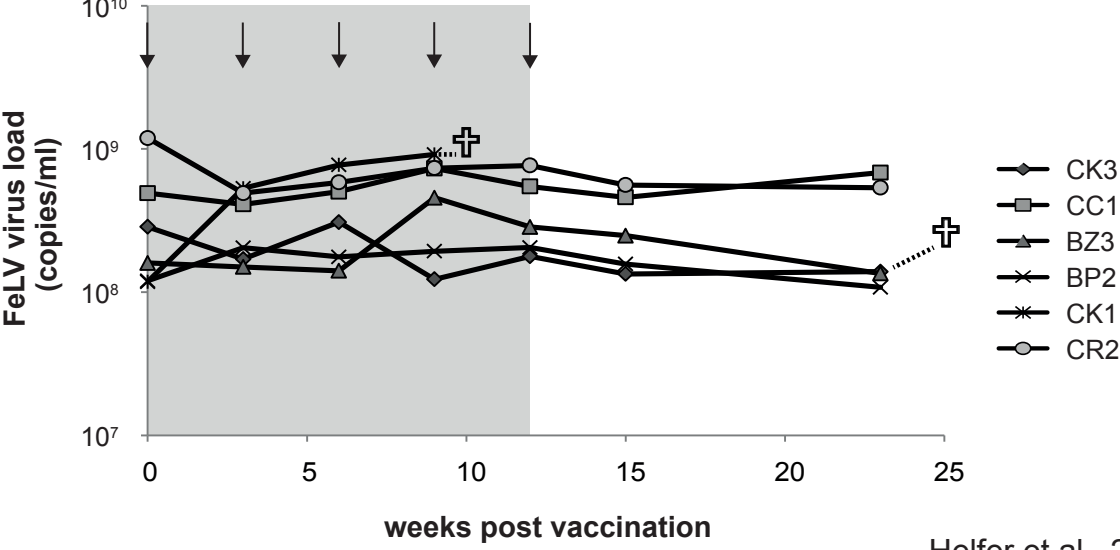
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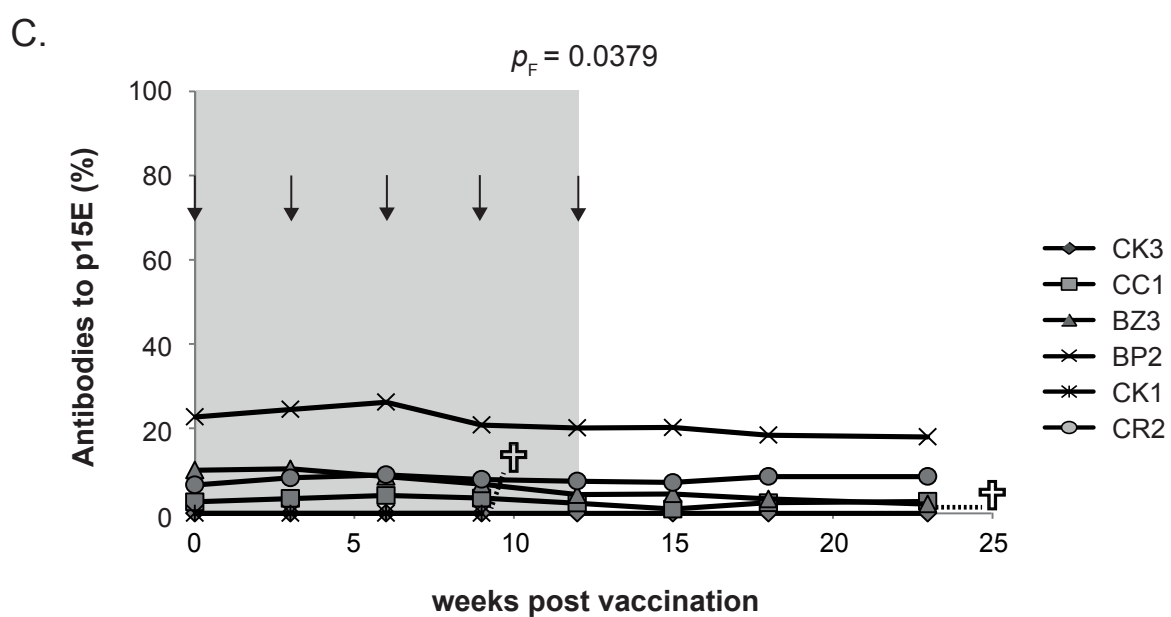
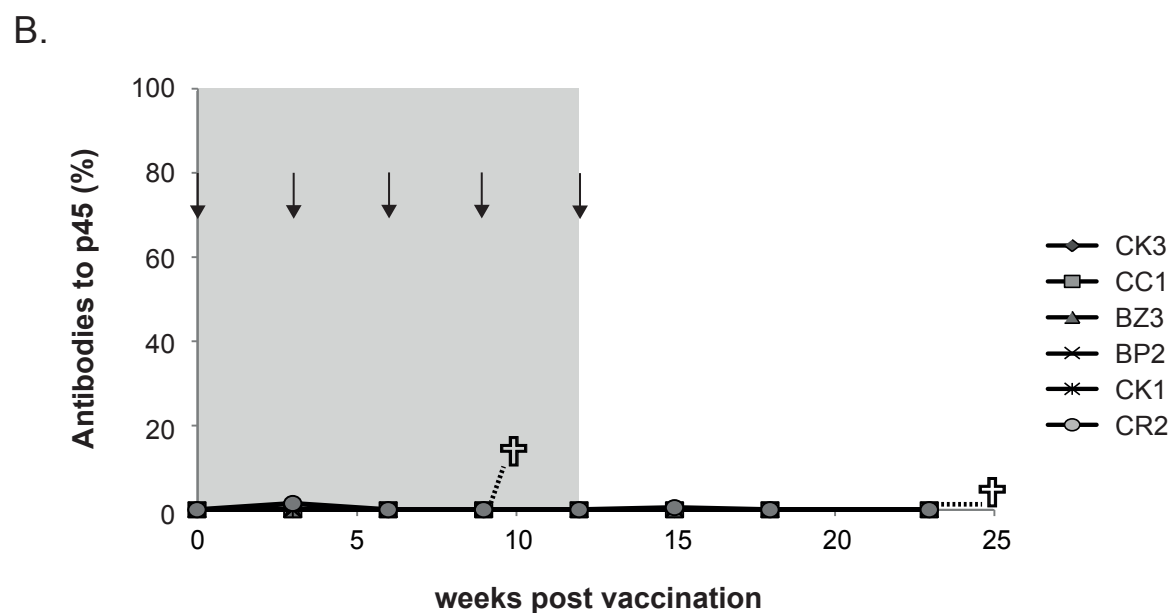
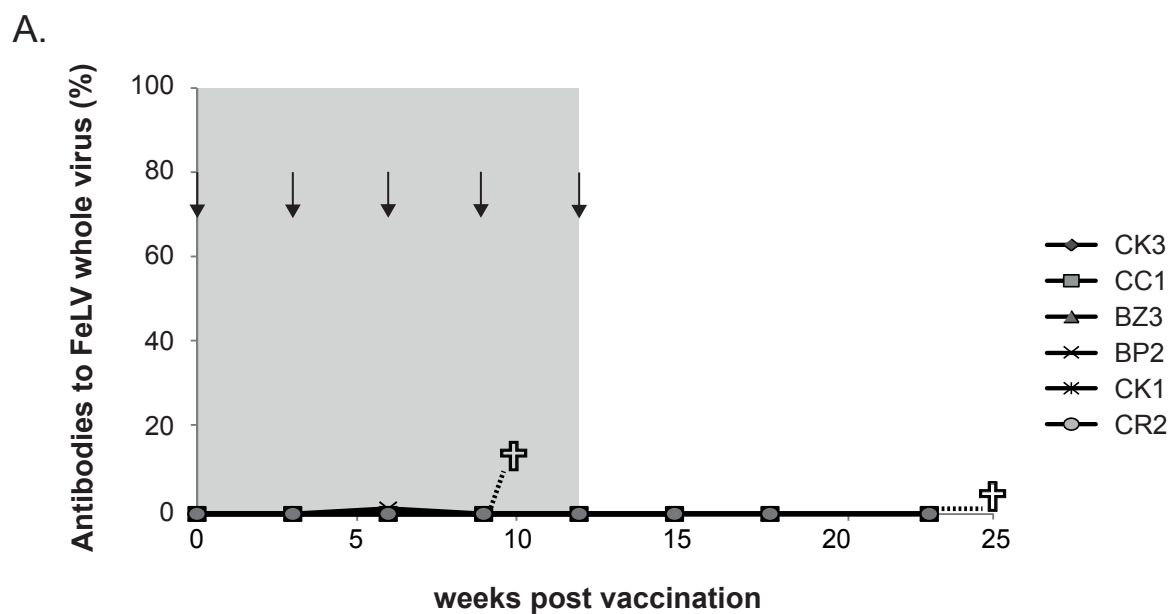


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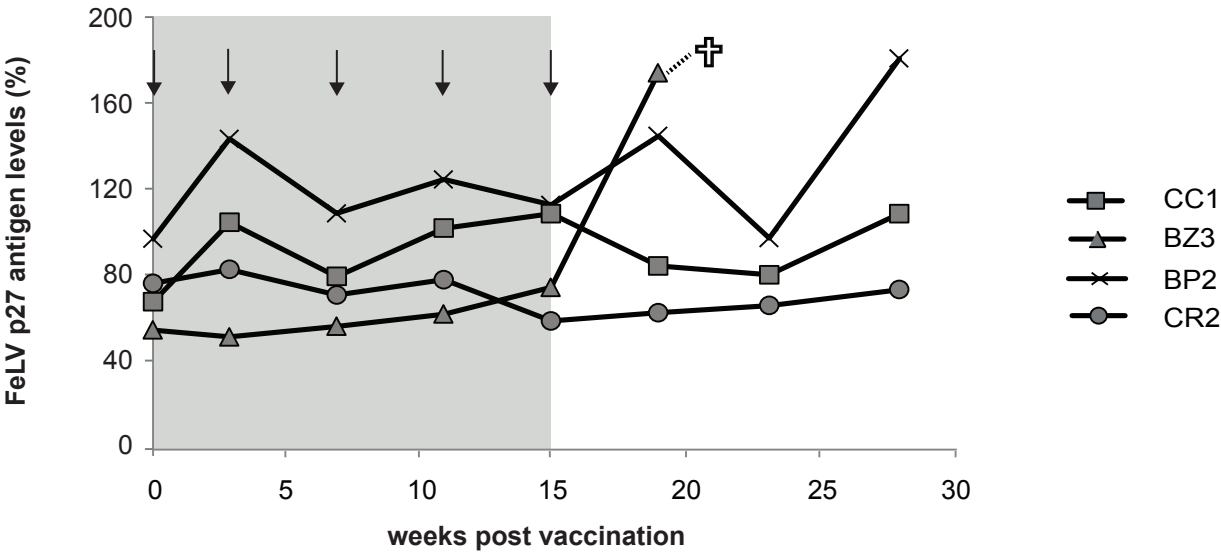


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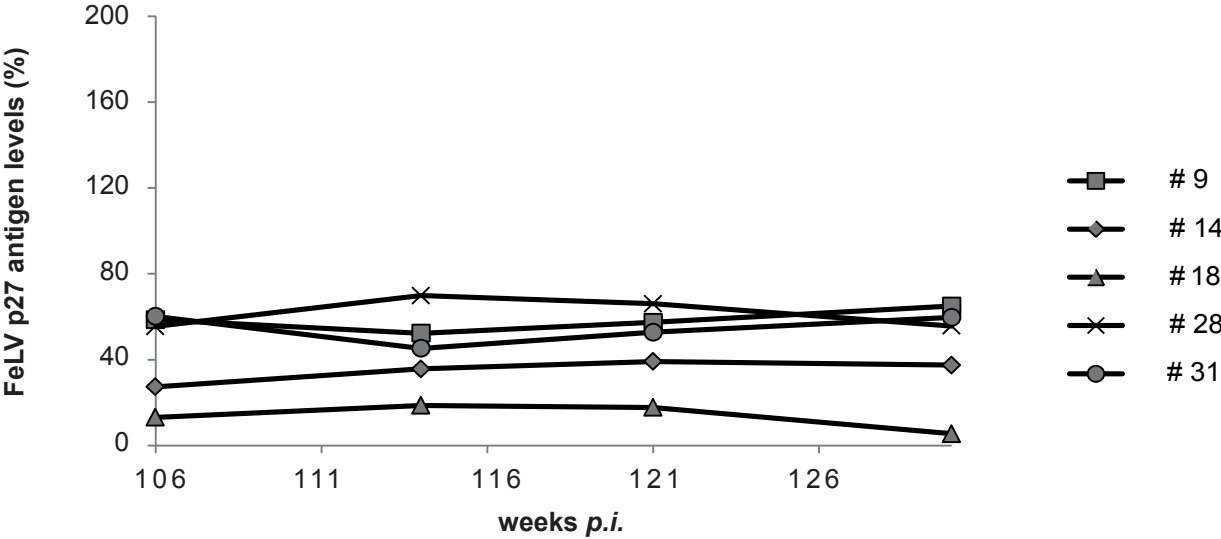




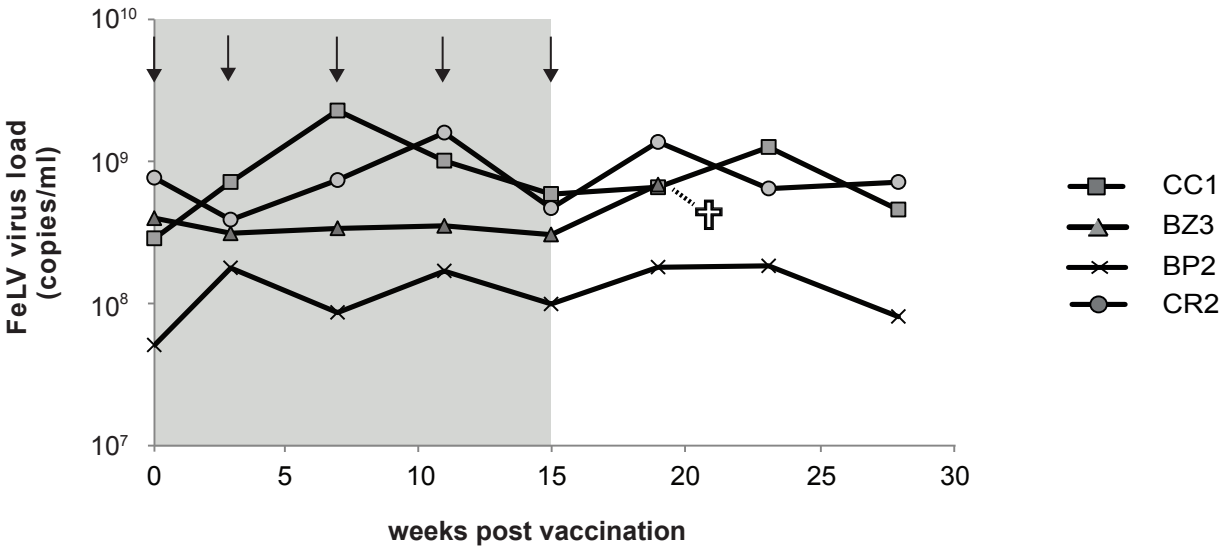
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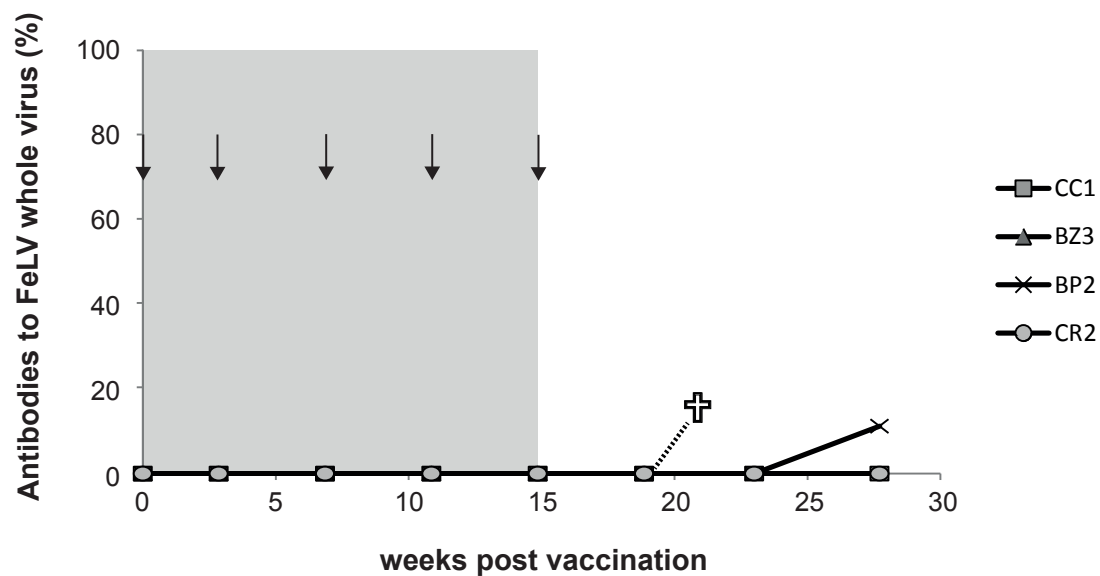
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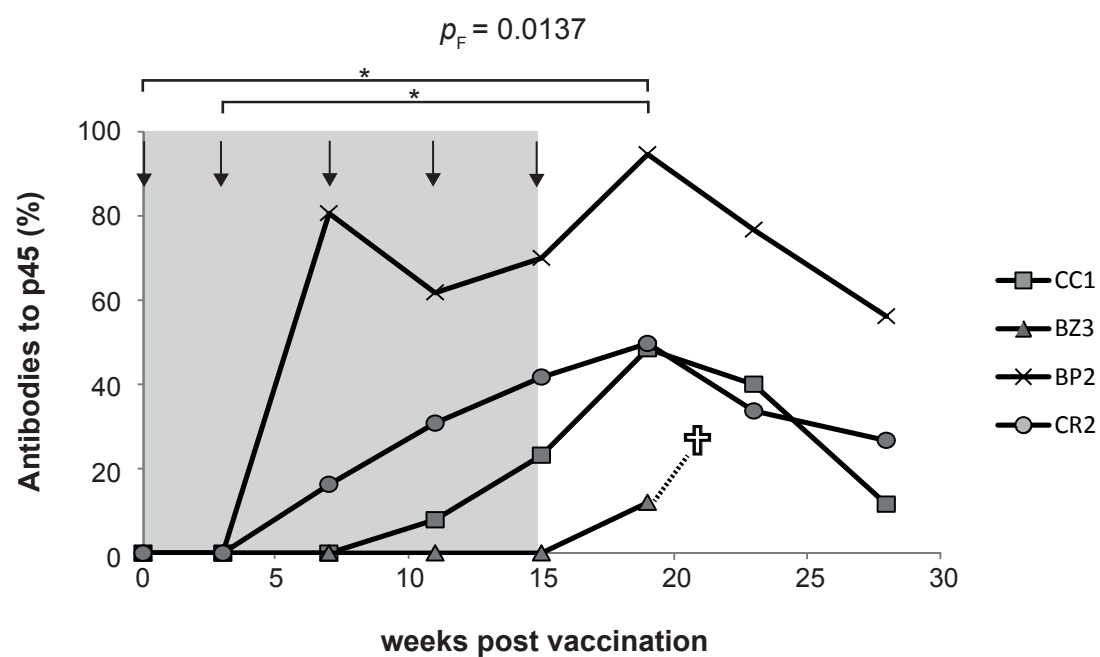
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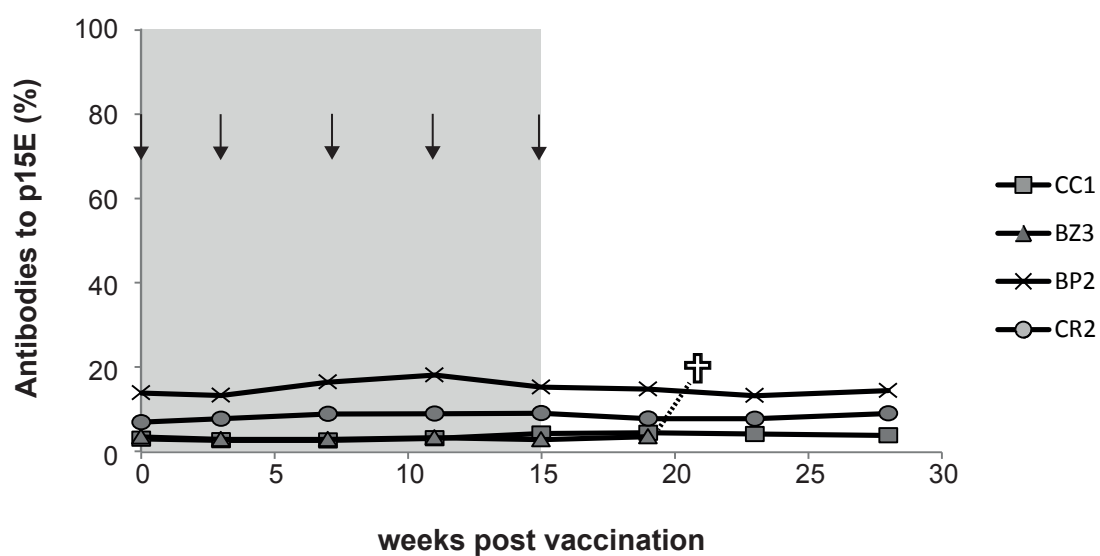
A.



B.



C.





## Supplemental Files

[Click here to download Supplemental Files: 14\\_08\\_07\\_Supplementary Figure 1\\_Purevax\\_Hmat.pdf](#)

## Supplemental Files

[Click here to download Supplemental Files: 14\\_08\\_07\\_Supplementary Figure 2\\_Purevax\\_Hmat.pdf](#)